Studies on Kitol I.
Preparation of Kitol from Whale liver Oil

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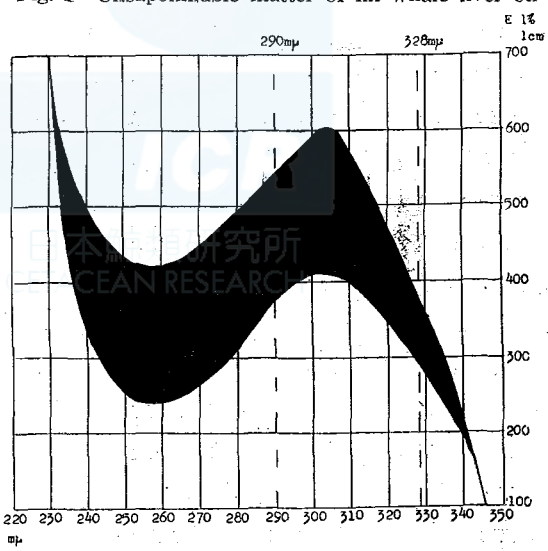
Willstaedt & Jensen[1] and Nakamiya[2] pointed out the presence of a Vitamin A-like substance, differing from the ordinary Vitamin A in its properties, in whale liver oil. Recently, Baxter, et al.[3] isolated kitol.

The presence of a foreign substance in whale liver oil had long been predicted by the difference of their spectrum analysis and the Carr-Price reaction from ordinary fish liver oil. The maximum of the absorption spectrum of the unsaponifiable matter of ordinary fish liver oil is in the neighborhood of 328 mμ, but that of whale liver oil is at 290—325 mμ, and the Carr-Price reaction of whale liver oil is bluish-purple whereas that of fish liver oil colors blue. From the proximity of the figures of extinction coefficient, $E^{1\%}_{1cm}$, of 265 mμ and 328 mμ in the absorption spectrum of the unsaponifiable matter of whale liver oil, it had been believed that the absorption curves would lie close together and overlap each other and an apparent maximum would lie in intermediate point.[2]

The authors also determined the extinction coefficient $E^{1\%}_{1cm}$ of the unsaponifiable matter of fin whale liver oil (Fig. 1)* by the spectrophotometer and were able to prove this point.

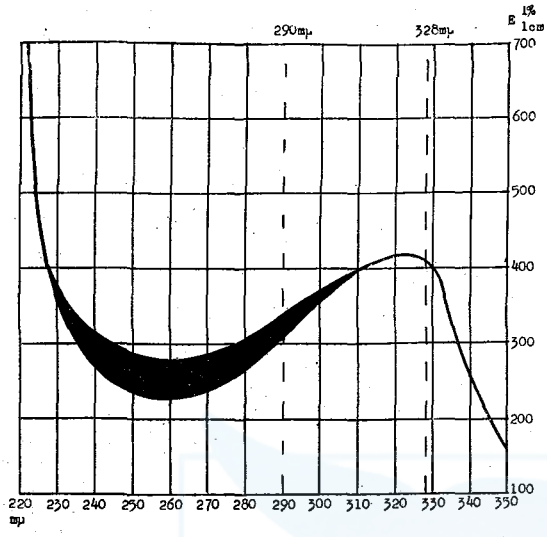
Fig. 1 Unsaponifiable matter of fin whale liver oil



This absorption curve apparently differs from that of tunny liver oil (Fig. 2). The greatest difference exists in the position of the absorption maximum and the width of the curve. The Carr-Price reaction of this unsaponifiable matter of

* The reason that the curve is so wide is because the absorption is so indistinct that the value of extinction coefficient, $E^{1\%}_{1cm}$, does not come out clearly.

Fig. 2 Unsaponifiable matter of tunny liver oil



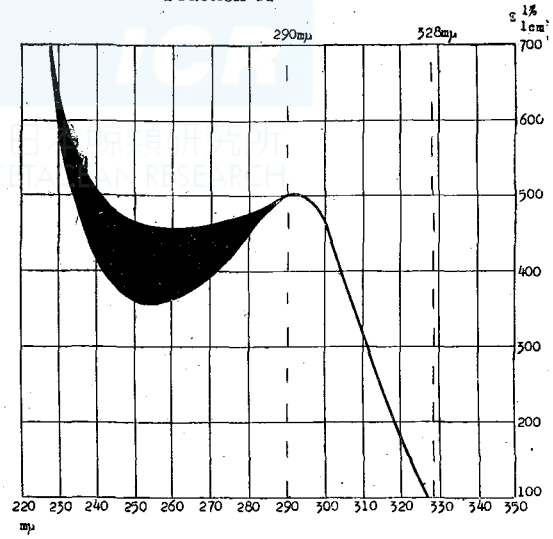
fin whale liver oil gives bluish purple color while that of tunny liver oil, blue.

The oxidation product of Vitamin A gives red or reddish brown coloring by this reaction. According to the authors' experiments, majority of this oxidation product transists into saponifiable matter during saponification so that it does not seem to constitute a reason for bluish purple coloration. Nakamiya and others harbored a doubt that it might be due to the large amount of cholesterol present but the authors take the view that this is due to the presence of kitol.

Kitol as isolated by Baxter, et al., were colorless, elongated prismatic crystals of Fp. 88—90°, with a molecular formula corresponding to $C_{40}H_{58}(OH)_2$, possessing 8 double bonds. It gives an absorption maximum at 290 mμ, and $E^{1\%}_{1cm}$ 290 mμ=707.

The fact that kitol possessed twice the molecular weight of Vitamin A was utilized in separating the two by the difference of solubility. The unsaponifiable matter of fin whale liver oil was dissolved in petroleum ether and this was extracted with 90% methanol into which Vitamin A was transited. The substance which remained in the petroleum ether solution to the last was examined by the spectrophotometer and the value of $E^{1\%}_{1cm}$ at each wave length was shown by a curve (Fig. 3). This substance showed an absorption maximum at 290 mμ, and the Carr-Price

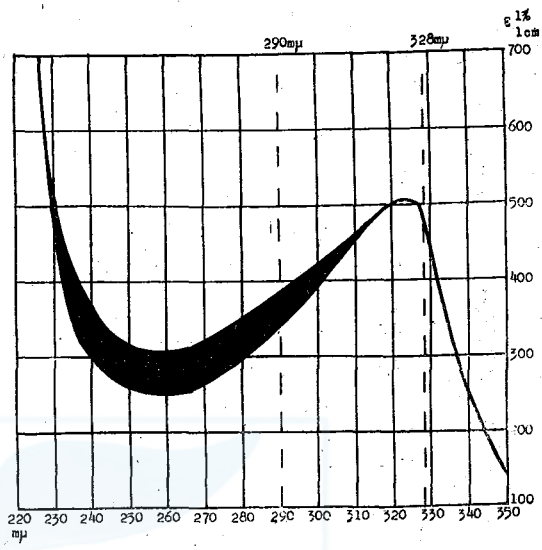
Fig. 3 Unsaponifiable matter of fin whale liver oil —Fraction A



reaction gives a red coloration. Apparently, it contained a very small amount of Vitamin A.

On the other hand the $E^{1\%}_{1cm}$ value of the substance that transited to 90% methanol (curve in Fig. 4) gave a maximum absorption at around 328 mμ,** which is very similar to the curve of tunny liver oil (Fig. 2). It does not give the indistinct curve as in Fig. 1, any more. The Carr-Price reaction is blue, so that this substance must be nothing but Vitamin A.

Fig. 4 Unsaponifiable matter of liver oil fin whale —Fraction B



From these facts, it can be assumed that the substance that reamained in petroleum ether to the last is clearly kitol.

EXPERIMENTAL

250 g. fin whale liver oil was warmed with 500 cc of 20% methanolic potash for 1 hour in a water bath. After saponification, this was extracted with ether and the ethereal layer was washed several times with water and dried with anhydrous Glauber's salt. After distilling off ether, 54 g. of unsaponifiable matter was obtained. This substance gives bluish purple coloration by Carr-Price reaction and, according to $E^{1\%}_{1cm}$ curve (Fig. 1) by spctrophotometer***, possesses an absorption maximum 303 mμ although the absorption area is so indistinct that it is hard to obtain a correct value of $E^{1\%}_{1cm}$. Accordingly, the curve became one of a wide band. This is apparently due to the fact that two or more substances are mixed in it and gives a complex absorption so that this must be taken as the apparent absorption maximum.

The total amount (54 g) of this unsuponifiable matter was dissolved in 1000 cc. of petroleum ether (Bp. 30—60°), placed in a separating funnel

** According to this experiment, the absorption maximum of Vitamin A-like substance isolated from the unsaponifiable matter of tunny liver and whale liver oil is more nearer 325 mμ than 328 mμ.

*** Spectrophotometer by Carl Zeiss was used. The solvent was absolute alcohol.

and extracted 38 times with 500 cc each 90% methanol. The petroleum ether layer was then washed several times with water, dried with anydrous Glauber's salt and distilled under reduced pressure. A reddish yellow, syrupy matter was obtained to the amount of 14 g. 90% Metanol solution was distilled under reduced pressure in carbon dioxide stream and the residue, composed mostly of water and a small amount of oil drops floating on it, was placed in a separating funnel. This was extracted with ether and the ethereal layer was distilled off after dry in with anhydrous Na_2SO_4. The residual matter, reddish yellow syrup obtained was ca. 39 g. About 1 g unsuponifiable matter was lost during this separating process.

The substance which transited to 90% methanol (hereafter designated as fraction B) gives a blue coloration to Carr-Price reaction, extinction coefficient curve as shown in Fig. 4 which approximately coincides with that (Fig. 2) of the unsuponifiable matter from tunny liver oil, i. e. it shows an absorption maximum at around 328 mμ and does not give the indistinct absorption as before separation. The Carr-Price reaction is not longer bluish purple but blue so that this is a substance devoid of foreign matter, i. e. Vitamin A alone.

The residual matter in petroleum ether (hereafter designated fraction A) gives a red coloratin to Carr-Price reaction and extinction coeffcient curve (Fig. 3) is clearly different from that of fraction B (Fig. 4) and possesses an absorption maximum at 290 mμ, $E^{1\%}_{1cm}$ 290 mμ=500.**** It is apparent that fraction A is kitol.

No presence of kitol were discovered in the oil obtained from the eyeball of sperm and sei whales.

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References

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**** Extinction coefficient of pure kitol at 290 mμ is 707[3] but that of kitol separated by Embree, etal.[4] at first was 580 at 290 mμ, which apparently was still quite impure. Frantion A obtained by the author theoretically contains 70% kitol.